parallel depending on the ions dwelling within the pore, but that (b) it is experimentally possible to dissociate the change in selectivity from that of stability, suggesting that the structural elements that determine either the selectivity or the stability of G_K are not identical. The functional and structural arguments that will be presented are not compatible with the notion of a rigid selectivity filter in which selectivity arises from protein structural elements alone.

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Biogenesis of Pore Architecture in Voltage-Gated K^+ Channels

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Although formation of the mature conducting pore of Kv channels necessitates tetramerization of individual subunits, it is possible that the first stages of pore formation occur early in biogenesis, prior to oligomerization of the transmembrane core. Our studies explore this hypothesis, specifically with respect to acquisition of secondary and tertiary structure. Using biogenic intermediates, a mass-tagging strategy, namely pegylation, and a molecular tape measure (Lu and Deutsch, 2005), we probed the pore domain of Kv1.3. The pore helix appears to form a compact secondary structure inside the ribosomal exit tunnel when located in the terminal 20Å of the tunnel whereas the turret is extended. Tertiary folding of the re-entrant pore loop was assessed by estimating pegylation rates of select cysteines engineered throughout the turret, the pore helix, and the loop preceding S6 in two monomeric constructs in the presence of ER membranes. The fastest rates were observed for turret and loop residues, whereas pore helix residues were 5-10 fold slower. To help interpret these observations, all-atom molecular dynamics simulations of a single monomer of the Kv1.2 channel pore domain were generated in a fully solvated lipid membrane. The two transmembrane helices S5 and S6 as well as the pore helix remain stable along the trajectory. These results are consistent with a tertiary re-entrant pore architecture being acquired at the monomer stage of Kv biogenesis, perhaps coincident with integration of transmembrane segments into the bilayer. [Supported by NIH grant GM 52302 and GM062342].
Reference: Lu and Deutsch, Biochemistry 44: 8230, 2005.

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Does a Single Mutation in the P-Loop Open a Novel Current Pathway Beside the Central A-Pore in the Human Voltage-Gated Potassium Channel Kv1.3

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Voltage-gated potassium channels are membrane proteins containing a potassium-selective ion conducting central α-pore. Recent studies showed that mutations in the voltage sensor of the *Shaker* channel can disclose another ion permeation pathway through the voltage sensing domain (S1-S4) beside the α-pore, the so called ω-pore described by Tombola *et al.* 2005 (*Neuron***45**:379-388). We investigated a mutant voltage-gated *h*Kv1.3 channel where the substitution of a cysteine in the pore-loop at position 388 (*Shaker* position 438) generated a current through the α-pore, and an inward-current at hyperpolarizing potentials carried by different cations (Li^+~Na^+>NH_4^+>Cs^+>K^+~Rb^+). This observed inward current appeared similar to the ω-current and was not affected by the α-pore blocker CTX, in contrary to the currents through the α-pore of the *h*Kv1.3_V388C mutant channel. Verapamil, which is acting from the intracellular side, could block both, the α-current with an IC_{50} of 3.5 µM and the observed inward-current at hyperpolarizing potentials with an IC_{50} of 2.3 µM in the *h*Kv1.3_V388C mutant channel. Due to the block of inward-current by verapamil we suppose that the observed inward-current runs through the verapamil binding site in the cavity between S6-S6 of two adjacent subunits in the *h*Kv1.3_V388C mutant channel. We hypothesize that the *h*Kv1.3_V388C mutation generated a channel with a second ion conducting pathway distinct from the α-pore, but not identical to the ω-pathway, running through one part of the *h*Kv1.3 verapamil binding site. The entry of the novel pathway is presumably located at the backside of Y395 (*Shaker* position 445), proceeds plane parallel to the α-pore, ending between S5 and S6 at the intracellular side of one α-subunit. Supported by grants from the 4SC AG (Martinsried) and the DFG (Gr848/14-1).

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Conductance and Concentration Relationship in a Reduced Model of the K Channel

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K^+ ions move rapidly through potassium channels more or less ignoring Na^+. The mechanism of selectivity is thought to depend on the solvation of K^+ and its electrostatic interactions with carbonyl dipoles of the channel wall, made of the side chains Thr Val Gly Tyr Gly TVGYG in many types of potassium channels. We calculate the conductance of the tetrameric KcsA prokaryotic K^+ channel measured in solutions of different K^+ concentration. The 3D model used here consists of two regions of different dielectric constant, one representing the protein and one representing a bath of implicit water. The geometry of the model is loosely based on the 'open' MthK crystal structure of Jiang's laboratory in which the intracellular half of the channel has a wide (~1.2 nm) pore radius. Ions are represented as hard spheres with Pauling radii. The surface charge on the protein is calculated using the induced charge computation method of Gillespie and collaborators. A Grand Canonical Monte Carlo approach developed by Boda maintains system neutrality while keeping bath concentrations fixed at values comparable to experiments. The Metropolis algorithm maintains a Boltzmann distribution to keep the system in thermodynamic equilibrium. The spatial density distribution of the ions allows an estimate of a characteristic slope conductance, for small driving force. Four pairs of GLU71/ASP80 ionizable residues lie directly behind the K^+ selectivity filter and have a substantial effect on potential energy profile along the selectivity filter. The model will be used to investigate the relationship of the protonation state of the residues, the composition of the bathing solutions, and the slope conductance.

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High-Resolution Structural Modeling of Ion Channel Pore-Forming Domains Using Rosetta

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Ion channels play a key role in many biological processes. Electrical signals conducted by ion channels are essential for rapid transmission of information in brain, cardiac muscle contraction, sensation of the environment, and secretion of hormones. Despite recent progress in determining the high-resolution structures of several members of ion channel family, the high-resolution structures of majority of ion channels remain unknown. We developed a novel approach to generate high-resolution structural models of pore-forming domains of ion channels using Rosetta symmetry-loop *de novo*/homology computational method. In this approach, the selectivity filter region residues were modeled using Rosetta *de novo* loop modeling method and the pore-forming transmembrane helices were modeled using Rosetta homology method. We tested our method on several known high-resolution ion channel structures, including KcsA (pdb: 1K4C and 1K4D), NaK (pdb: 3E86), and Kv1.2 (pdb: 2R9R) channels. Results show that Rosetta symmetry-loop modeling method is able to predict the selectivity filter region of ion channels with high-resolution (1.0-1.5 Å root mean square deviation of backbone atoms from the native structure) and that Rosetta full-atom scoring function is able to discriminate well the best models in the majority of tested cases. Our results show that the Rosetta symmetry-loop *de novo*/homology modeling method is a powerful new approach for high-resolution structure prediction of pore-forming domains of ion channels. Supported by University of Washington Royalty Research Fund grant (to V.Y.-Y), HHMI grant (to D.B.), and NIH Grant R01 NS15751 (to W.A.C.).

615-Pos

Can Bubble Gates Be Seen in Experiments?

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Bubble gating is the hypothesis that the flux through an ion channel can be controlled by the formation or breaking of a bubble in a narrow hydrophobic gate [1]. If a bubble forms, liquid water is transformed into the gas phase and ions face a large dielectric boundary, which stops the flux through the gate.
The physics model of the bubble gate [1] allows one to systematically look for experimentally verifiable signs of bubbles. Here I discuss two possibilities.
1. Bubble formation is a small scale pseudo phase transition form a liquid into a gas. A liquid has a large internal energy, but a small entropy, while the gas has a large entropy, but a small internal energy. When a bubble forms, the free energy changes only slightly, because the large change in internal energy is almost balanced by a large change in entropy.
2. Hydrophobic gases, such as nobel gases, have a small solubility in water. If a bubble forms, it is filled by the hydrophobic gas [2].
[1] R. Roth and K.M. Kroll, J. Phys.: Condens. Matter 16, 6517-6530 (2006).
[2] R. Roth, D. Gillespie, W. Nonner, and R.E. Eisenberg, Biophys. J. 94, 4282-4298 (2008).